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Scapuloperoneal syndrome type Kaeser and a wide phenotypic spectrum of adult-onset, dominant myopathies are associated with the desmin mutation R350P

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In 1965, an adult-onset, autosomal dominant disorder with a peculiar scapuloperoneal distribution of weakness and atrophy was described in a large, multi-generation kindred and named 'scapuloperoneal syndrome type Kaeser' (OMIM #181400). By genetic analysis of the original kindred, we discovered a heterozygous missense mutation of the desmin gene (R350P) cosegregating with the disorder. Moreover, we detected *DES* R350P in four unrelated German families allowing for genotype–phenotype correlations in a total of 15 patients carrying the same mutation. Large clinical variability was recognized, even within the same family, ranging from scapuloperoneal ($n = 2$, 12%), limb girdle ($n = 10$, 60%) and distal phenotypes ($n = 3$, 18%) with variable cardiac ($n = 7$, 41%) or respiratory involvement ($n = 7$, 41%). Facial weakness, dysphagia and gynaecomastia were frequent additional symptoms. Overall and within each family, affected men seemingly bear a higher risk of sudden, cardiac death as compared to affected women. Moreover, histological and immunohistochemical examination of muscle biopsy specimens revealed a wide spectrum of findings ranging from near normal or unspecific pathology to typical, myofibrillar changes with accumulation of desmin. This study reveals that the clinical and pathological variability generally observed in desminopathies may not be attributed to the nature of the *DES* mutation alone, but may be influenced by additional genetic and epigenetic factors such as gender. In addition, mutations of the desmin gene should be considered early in the diagnostic work-up of any adult-onset, dominant myopathy, even if specific myofibrillar pathology is absent.

Keywords: myofibrillar myopathy; scapuloperoneal syndrome; desminopathy; desmin-related myopathy

Abbreviations: EM = electron microscopy; EMG = electromyography; Gd-DTPA = gadolinium-diethyltriaminepentaacetic acid; LGMD = limb girdle muscular dystrophy; MRI = magnetic resonance imaging

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Introduction

Mutations of the human desmin gene on chromosome 2q35 cause familial or sporadic forms of skeletal myopathy, characterized morphologically by abnormal accumulation of desmin within muscle fibres (Goebel, 1995). The majority of cases show autosomal-dominant inheritance, but rare autosomal-recessive cases as well as an increasing number of sporadic cases have been reported (Goldfarb *et al.*, 2004). Several clinical presentations such as rare severe childhood-onset cardioskeletal myopathy (Goldfarb *et al.*, 1998), adult-onset skeletal myopathy with cardiac involvement (Goldfarb *et al.*, 1998; Park *et al.*, 2000*a*, *b*; Kaminska *et al.*, 2004), skeletal myopathy without cardiac involvement

(Kaminska *et al.*, 2004), severe generalized myopathy (Ariza *et al.*, 1995), pure dilated cardiomyopathy (Li *et al.*, 1999), cardiomyopathy with distal weakness (Sugawara *et al.*, 2000; Goudeau *et al.*, 2001; Schroder *et al.*, 2003) and distal myopathy with cardiac, respiratory, bulbar and facial involvement (Horowitz and Schmalbruch, 1994; Sjoberg *et al.*, 1999) were described (OMIM #125660). Most *DES* mutations were reported in one family or in a few patients, only. Therefore, it is difficult to assess whether a distinct clinical phenotype is closely correlated to a certain mutation or whether certain mutations cause various clinical presentations.

Recently, we identified a novel *DES* mutation (R350P) in a German family with distal myopathy. R350P was shown to exert a dominant negative effect on the ordered lateral arrangement of desmin subunits leading to abnormal protein aggregation. Here we present clinical findings in 15 patients from 5 unrelated families harbouring *DES* (R350P) and demonstrate a wide phenotypic spectrum associated with this mutation including scapuloperoneal syndrome type Kaeser (OMIM #181400) (Kaeser, 1965).

Material and methods

Patients

A total of 205 unrelated, adult patients with clinical evidence for a myopathy in scapuloperoneal, distal or limb girdle distribution were screened for mutations in the desmin gene (see later). *DES* (R350P) was detected in five families. All index patients and affected relatives described in this study were examined by one of the coauthors. Consanguinity was not reported. Pedigrees were compatible with autosomal dominant traits. All patients described are of German descent.

Magnetic resonance imaging

A recently introduced 1.5 tesla scanner (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany) combines 76 coil elements ('matrix coils') and 32 receiver channels. It allows whole body MRI in all three dimensions with free table movement. The protocol used for musculoskeletal system on this scanner incorporates T1-weighted SE (TR 540, TE 13) before and after intravenous application of Gd-DTPA as contrast-material and STIR-sequences (TR 2680, TE 101 and TI 150).

Muscle biopsy

An open muscle biopsy was taken from the right vastus lateralis muscle of patient 9 and from the left gastrocnemius muscle of patient 13 in family 4 and analysed by standard histological and immunohistological methods. Protein aggregates were visualized using antibodies against desmin (Dako M0760, clone D33, Glostrup, Denmark; dilution 1 : 100), αB-crystallin (Novocastra, Newcastle Upon Tyne, UK, dilution 1 : 100) and filamin c (clone RR90; dilution 1 : 3 (van der Ven *et al.*, 2000) and appropriate secondary antibodies for immunohistochemistry.

Haplotype analysis

Venous blood samples for genomic DNA extraction were collected with informed consent. Haplotype analysis was performed using polymorphic microsatellite markers for the chromosomal loci of *LGMD1A–F*, *MYH2A*, *CRYAB* and *DES* as recently described (von der Hagen *et al.*, 2006). For each gene locus, four to six microsatellite markers flanking the gene on either side were investigated. The order of markers was based on published human linkage maps and physical mapping data.

Mutation analysis of the desmin gene (*DES*)

A PCR fragment encompassing the mutation R350P was amplified by PCR using primers D6f [5′-ATGGCCAGGACCTGACCATT CTG-3′] and D350r [5′-TGGCAATCTCCACATCCAGGGCC-3′]. The resulting 293 bp fragment was purified using the NucleoSpin Extract kit (Macherey–Nagel, Düren, Germany), digested with the restriction endonuclease *Hpa*II (New England Biolabs, Frankfurt, Germany), and the resulting fragments separated on 2% agarose gels by electrophoresis. The mutant fragment (R350P) digested into three fragments of 131, 88 and 74 bp, whereas wild-type DNA is cleaved into two fragments of 205 and 88 bp length. To confirm the presence of *DES* R350P the same PCR product was sequenced using an Applied Biosystems model 3100 Avant DNA sequencer and fluorescein-labelled dideoxy terminators (Perkin–Elmer, Foster City, CA, USA).

Results

Molecular genetics

Linkage analysis was performed in family 1 and 2, and suggested possible linkage to the *DES* gene, whereas loci for *LGMD1A–F*, *MYH2A* and *CRYAB* were excluded. Mutation screening of *DES* revealed a heterozygous R350P (1049 G > C) mutation in all affected family members, segregating with the disease phenotype. The mutation has been previously described by us in family 4 (Bar *et al.*, 2005). By screening a cohort of 205 patients, the same mutation was identified in two additional, unrelated families (families 3 and 5), where it also segregates with the disease. Haplotype analysis using six microsatellite markers flanking the desmin gene revealed that the affected family members in families 1–5 share a common haplotype on one allele including the gene region which cosegregates with the R350P mutation (Table 1). In families 3, 4 and 5, recombination events occurred downstream from the *DES* gene region between markers *D2S2359* and *D2S126* and between *D2S163* and *D2S2359*, respectively.

Interestingly, the disease allele in all five families shares a core region of at least 3 Mb with most distal common microsatellite marker D2S163 (Table 1). This may point towards a common origin of the mutation (founder allele).

Clinical observations

Family 1—scapuloperoneal phenotype

We examined two affected descendants of the kindred originally described by Kaeser (1965) with 12 affected members in five generations following autosomal dominant inheritance, and a distinct scapuloperoneal distribution of weakness (Fig. 1). The 60-year-old male index patient (patient 1, Table 2, Figs 2, 3 and 4) experienced first

Table 1 Haplotypes of the index patients

Marker	Type	Location (bp)	Fam 1		Fam 2		Fam 3		Fam 4		Fam 5	
			Pat 1/VI : 59		Pat 3/V : 4		Pat 8/IV : 2		Pat 9/II : 3		Pat 15/III : 2	
D2SI64	MS	217 568 095	4	3	4	2	4	2	4	1	4	3
D2S2249	MS	218 323 386	2	4	2	1	2	4	2	3	2	3
D2S2250	MS	219 365 196	2	1	2	2	2	3	2	1	2	3
c.1049G>C (p.R350P)	Mut.	219 994 327	C	G	C	G	C	G	C	G	C	G
D2SI63	MS	220 400 516	3	2	3	1	3	1	3	2	3	3
D2S2359	MS	220 553 981	2	1	2	3	2	1	3	4	3	3
D2SI26	MS	221 625 158	5	4	5	1	1	2	2	2	3	5

Haplotype analysis of five unrelated R350P patients using six microsatellite markers (MS) flanking the desmin gene and one single nucleotide polymorphism (SNP). Vertical columns represent individual haplotypes of the analysed patients, whereas each row represents the genotypes of the MS and the SNP. The markers are listed from centromeric to telomeric; the mutation [1049G>C (R350P)] is shown in the middle. Genomic locations are from the UCSC reference database via http://www.genome.ucsc.edu/. The haplotype cosegregating with the R350P mutation has been (representing a putative founder haplotype), while a differing genotype has been retained white. All patients share a common haplotype stretching from marker D2SI64 to D2SI63 including the *DES* gene region.

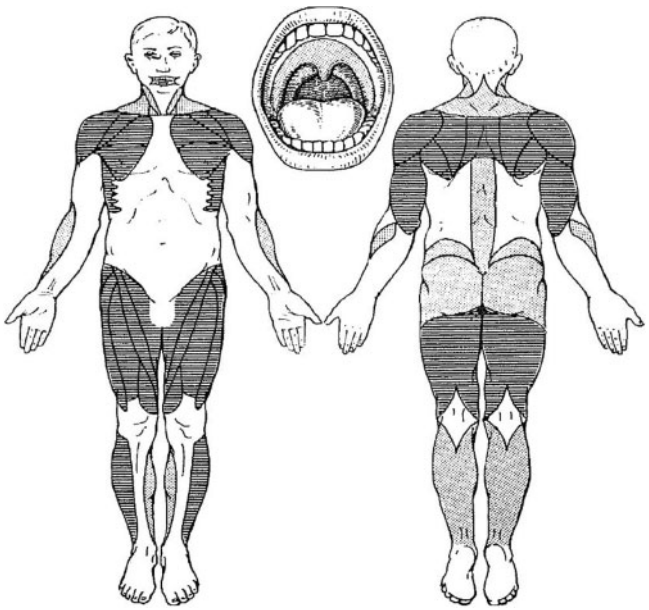

Fig. 1 Distribution of muscle weakness (from: Kaeser, 1965) Black: severe weakness/grey: moderate weakness.

walking difficulties at age 39 years. At age 41 years, paresis of foot flexors and extensors was noted, and at age 43 years, shoulder girdle weakness occurred. Ten years after onset of the disease, the patient became wheelchair-bound; standing with support was possible up to age 58 years. At examination at age 60 years, the patient showed pronounced atrophy and weakness of the lower limb and shoulder girdle muscles as well as a mild involvement of the facial muscles, dysphagia and gynaecomastia. Whole body MRI scan showed marked atrophy and fatty degeneration of proximal and distal muscles, only the biceps brachii muscle was relatively spared (Fig. 3). CK levels were elevated up to 700 U/l (normal value <80). There was subclinical cardiac involvement with mild arrhythmia. Genetically, facioscapulohumeral muscular dystrophy (FSHD) was excluded. EMG showed myogenic findings in various muscles as well as subtle neurogenic changes in the distal lower extremities. Morphological and ultrastructural examination of the biceps brachii muscle revealed unspecific signs of myopathy. Immunohistochemical staining for desmin and filamin c was normal except for a few, subsarcolemmal aggregates in single fibres (Fig. 5).

The 61-year-old sister of the index patient (patient 2, Table 2) observed mild abdominal weakness between age 40 and 50 years. At age 56 years, mild dysphagia and weakness of foot extensors occurred. Since age 60 years, she noticed marked problems in climbing stairs or in getting up from sitting position due to proximal leg weakness. Two years later, distal and proximal weakness of arm muscles developed, and dysphagia worsened. CK levels were only elevated at 215 U/l (normal value <167). There is no cardiac or pulmonary involvement, and the patient is still able to walk without aids for up to 30 min.

Historical data of the family reveal mean onset of disease around 40 years of age with earlier onset in male as compared to female patients. Up to now, 20 persons (11 males, 9 females) from seven generations are reported to be affected, penetrance seems to be complete.

Family 2—limb girdle phenotype

In this family, we examined five patients from two generations with an autosomal dominant myopathy. The female index patient (patient 3, Table 2, Fig. 4) showed first symptoms at age 50 years with slowly progressive proximal leg weakness and cardiac conduction problems. During the progression of the disease, distal involvement occurred in leg muscles. However, only mild proximal paresis was seen in the upper extremity, and no dysphagia. Since age 70 years, there is severe pulmonary involvement requiring non-invasive ventilation. CK levels were normal or mildly elevated. EMG showed a mixed myopathic–neurogenic pattern, muscle biopsy revealed a degenerative myopathy with additional neurogenic changes, fibre-size variations, rimmed vacuoles, whorled fibres, suggesting myofibrillar myopathy. Additional family

Table 2 Clinical, morphological and genetic findings in R350P desminopathy patients

Patient no./ pedigree no.	Age (years)	Sex	Age onset	Initial symptoms	Distribution of weakness	MRC grades (Arms.proximal Arms.distal Legs.proximal Legs.distal)	Additional signs and symptoms	CK (U/l)	EMG	Histology	RV	Protein aggregates/ desmin positive inclusions	EM
Family 1													
I/VI : 59	60	M	39	Climbing stairs impaired	Scapuloperoneal, wheel-chair bound	3–4/5 4–5/5 2–3/5 1–2/5	Dysphagia, gynaecomastia, cardiac (mild arrhythmia), mild facial weakness	700	Mixed myopathic/ neurogenic	Mild degenerative myopathy	–	(+)	Z-line disorganization no protein aggregates
2/VI : 58	61	F	40	Abdominal weakness, foot drop, dysphagia	Scapuloperoneal, still walks independently	4–5/5 5/5 4/5 4/5	Dysphagia, facial weakness	215	n.d.	n.d.	n.d.	n.d.	n.d.
Family 2													
3/V : 4	82	F	50	Climbing stairs impaired	LGMD, bed-confined	3–4/5 4/5 1–2/5 4/5	Cardiac (conduction problems), pulmonary (requiring ventilation)	150	Mainly myopathic	Degenerative myopathy with neurogenic-like changes	–	n.d	n.d.
4/VI : 15	46	M	30	Myalgia in proximal leg muscles	LGMD, walks independently	5/5 5/5 4/5 5/5	None	400	Normal	n.d.	n.d.	n.d.	n.d.
5/VI : 7	†60	M	35	Climbing stairs impaired	LGMD, wheel-chair bound	2–3/5 2–3/5 1–2/5 1–2/5	Fatal respiratory failure at age 60	630	Mixed myopathic/ neurogenic	Neurogenic-like atrophy	+	n.d	n.d.
6/VII : 7	41	M	38	Myalgia in proximal leg muscles	LGMD, walks independently	5/5 5/5 4/5 5/5	None	200	Normal	n.d.	n.d.	n.d.	n.d.
7/VII : 25	52	M	40	Standing on heels and toes impaired	Distal/LGMD, walks with support	4/5 5/5 3–4/5 3/5	None	500	Myopathic	Degenerative myopathy	++	n.d.	n.d
Family 3													
8/IV : 2	48	M	31	Walking impaired due to foot drop	Distal, walks independently	4–5/5 5/5 4/5 2/5	Mild dysphagia, gynaecomastia	900	Myopathic	Degenerative myopathy with neurogenic-like changes	+	Normal	Z-line disorganization autophagic vacuoles no protein aggregates

(*continued*)

Table 2 Continued

Patient no./ pedigree no.	Age (years)	Sex	Age onset	Initial symptoms	Distribution of weakness	MRC grades (Arms.proximal Arms.distal Legs.proximal Legs.distal)	Additional signs and symptoms	CK (U/l)	EMG	Histology	RV	Protein aggregates/ desmin positive inclusions	EM
Family 4													
9/II : 3	56	M	48	Dyspnoea on exertion	LGMD, proximal and distal weakness in upper and lower extremities, walks independently	4/5 5/5 3/5 4/5	Respiratory insufficiency (requiring ventilation), cardiac (arrhythmia), deafness, gynaecomastia	2580	Myopathic	Degenerative myopathy	+	+	Z-line streaming massive granulofilamentous material
10/I : 2	†44	F	n.d.	Proximal leg weakness	LGMD	n.d.	Sudden cardiac death at age 44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
11/II : 5	50	F	44	Dyspnoea on exertion, proximal leg weakness	LGMD, proximal and distal weakness in lower extremities, walks independently	5/5 5/5 4/5 4/5	Intermittent palpitations, exertional dyspnoea	370	n.d.	n.d.	n.d.	n.d.	n.d.
12/II : 1	†46	M	31	Proximal leg weakness	LGMD, proximal weakness in lower extremities	n.d.	Acute cardiorespiratory failure at age 46	470	n.d.	n.d.	n.d.	n.d.	n.d.
13/III : 1	26	M	–	No complaints	Distal weakness in lower extremities, walks independently	5/5 5/5 5/5 4/5	Deafness	540	Mixed myopathic/ neurogenic	Degenerative myopathy with neurogenic-like changes	+	+	n.d
Family 5										n.d.	n.d.	n.d.	n.d.
14/IV : 3	33	F	30	Myalgia, climbing stairs impaired	LGMD, proximal and distal weakness in lower extremities, walks independently	5/5 5/5 4/5 3/5	Beginning respiratory involvement	452	Myopathic	n.d.	n.d.	n.d.	n.d.
15/III 0 : 2	64	F	40	Proximal leg weakness, foot drop	LGMD, wheelchair-bound	3/5 4/5 2–3/5 2/5	Respiratory insufficiency (requiring ventilation), cardiac (arrhythmia)	108	Myopathic	Degenerative myopathy	+	n.d.	n.d.

n.d. = not done; + = some; ++ = many; − = none; CK = creatine kinase; LGMD = limb girdle muscular dystrophy; EMG = electromyography; EM = electron microscopy; RV = rimmed vacuoles; † = deceased.

Fig. 2 Clinical picture of patient I. Note the scapuloperoneal pattern of weakness along with gynaecomastia.

members (patients 4–7, Table 2) were examined and found to be similarly affected with a limb girdle distribution of weakness. Similar to family 1, male patients experienced an earlier onset and a more severe course of the disease.

Historical data on the family revealed, that 18 patients (13 males, 7 females) in seven generations were known to be affected. Assignment (affected/unaffected) for generations II, III and IV (date of birth around 1780–1900) may be imperfect, given that a mild clinical presentation at a higher age may have been missed. Overall, patients show a limb girdle phenotype, frequently with pronounced cardiac involvement, and four affected males died from sudden cardiac events. Furthermore, two affected family members (one male, one female) show marked pulmonary involvement requiring non-invasive ventilation.

Family 3—distal myopathy phenotype

First symptoms occurred at age 31 years in the now 50-year-old male index patient (patient 8, Table 2, Fig. 4) as distal leg weakness predominantly in foot extensors. Ten years later, abdominal weakness, mildly progressive proximal leg weakness and gynaecomastia were noted. Since age 46 years, dysphagia and proximal arm weakness additionally occurred, but up to now we observed no signs of cardiac or pulmonary impairment. CK levels were elevated between 300 and 1000 U/l (normal value <180). Histologically, a myopathy with rimmed vacuoles was diagnosed; Z-line disorganization and autophagic vacuoles were observed by electron microscopy. Whole body MRI scan showed symmetric atrophy and degeneration of deltoideus muscles, while biceps brachii, supra- and infraspinatus muscles are relatively spared (Fig. 3). Furthermore, there was marked involvement of trunk muscles, proximal and distal lower-limb muscles with predominance of fatty degeneration in tibialis anterior muscles. Six individuals (three males, three females) from four generations are known to be affected.

Family 4—mixed distal myopathy/limb girdle phenotype

This family harbouring the R350P mutation has been recently described by us (Bar *et al.*, 2005) and re-examined for the purpose of this study. The index patient is a 56-year-old male presenting with a history of increasing dyspnoea on exertion starting in his mid-forties (patient 9, Table 2, Fig. 4). Neuromuscular symptoms with difficulty in lifting his arms over shoulder level were first noted at age 48 years. Neurological examination showed mild bilateral weakness of proximal arm and shoulder girdle muscles and moderate weakness of both pelvic and proximal leg muscles. In addition, he exhibited mild weakness of distal leg muscles and showed mild gynaecomastia. Respiratory and cardiac work-up showed a restrictive ventilation disorder and a cardiomyopathy with conduction disorder. EMG showed a myopathic pattern, nerve conduction velocity was normal. Biopsy of his vastus lateralis muscle revealed myopathic findings including increased fibre-size variation, internalized myonuclei, rare angulated atrophic fibres, multiple fibres with cytoplasmic and subsarcolemmal basophilic inclusions and conspicuous cytoplasmic protein aggregates in a large number of myofibres. These aggregates were immunoreactive for desmin and αB-crystallin (Fig. 5).

Several other family members were similarly affected (patients 10–13, Table 2). His mother suffered from slowly progressive leg weakness and died at age 44 years because of acute cardiac failure (patient 10, Table 2). His 50-year-old sister reported progressive muscle weakness and dyspnoea on exertion, starting at age 44 years. Her neurological examination demonstrated moderate weakness of distal and proximal leg muscles (patient 11, Table 2). His brother suffered from proximal leg weakness starting at age 31 years and died at age 46 years because of progressive cardiorespiratory insufficiency (patient 12, Table 2). One out of four offspring of patient 12 showed mild weakness of foot extensor muscles (patient 13 in Table 2). Muscle biopsy taken from the gastrocnemius muscle revealed a myopathic

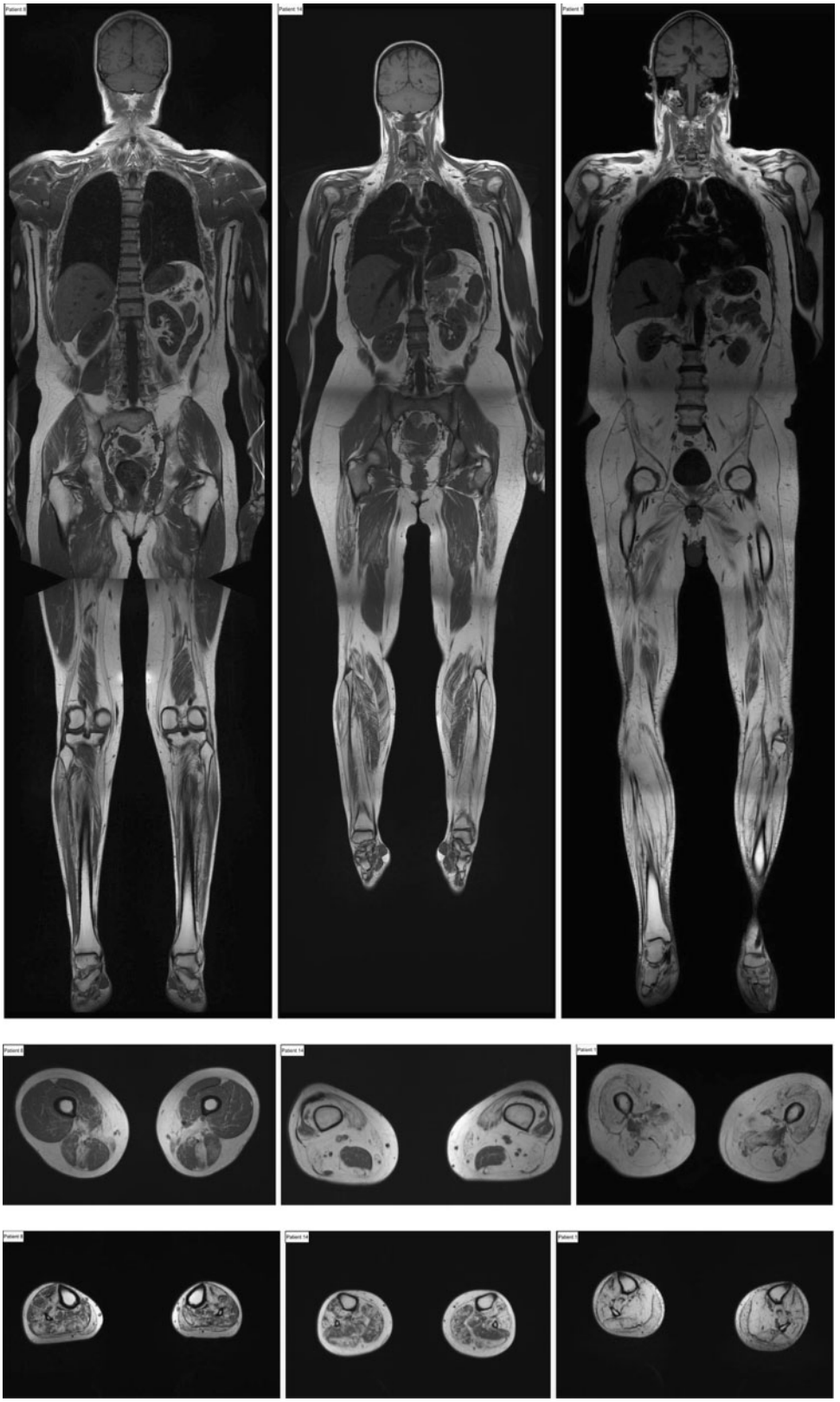

Fig. 3 Whole body MRI scans (T1-weighted images) and transverse sections of upper and lower-limb muscles. Patient 8: (left) symmetric atrophy and degeneration of deltoideus muscles, while biceps brachii, supra- and infraspinatus muscles are relatively spared. Note the marked involvement of trunk muscles, proximal and distal lower-limb muscles with predominance of fatty degeneration in tibialis anterior muscles. Patient14: (middle) marked atrophy and fatty degeneration of proximal and distal legmuscles, predominantly in the dorsal leg compartment, while in the upper extremities only deltoideus muscles are mildly affected. Patient 1: (right) marked atrophy and fatty degeneration of proximal and distal muscles, only the biceps brachii muscle is relatively spared.

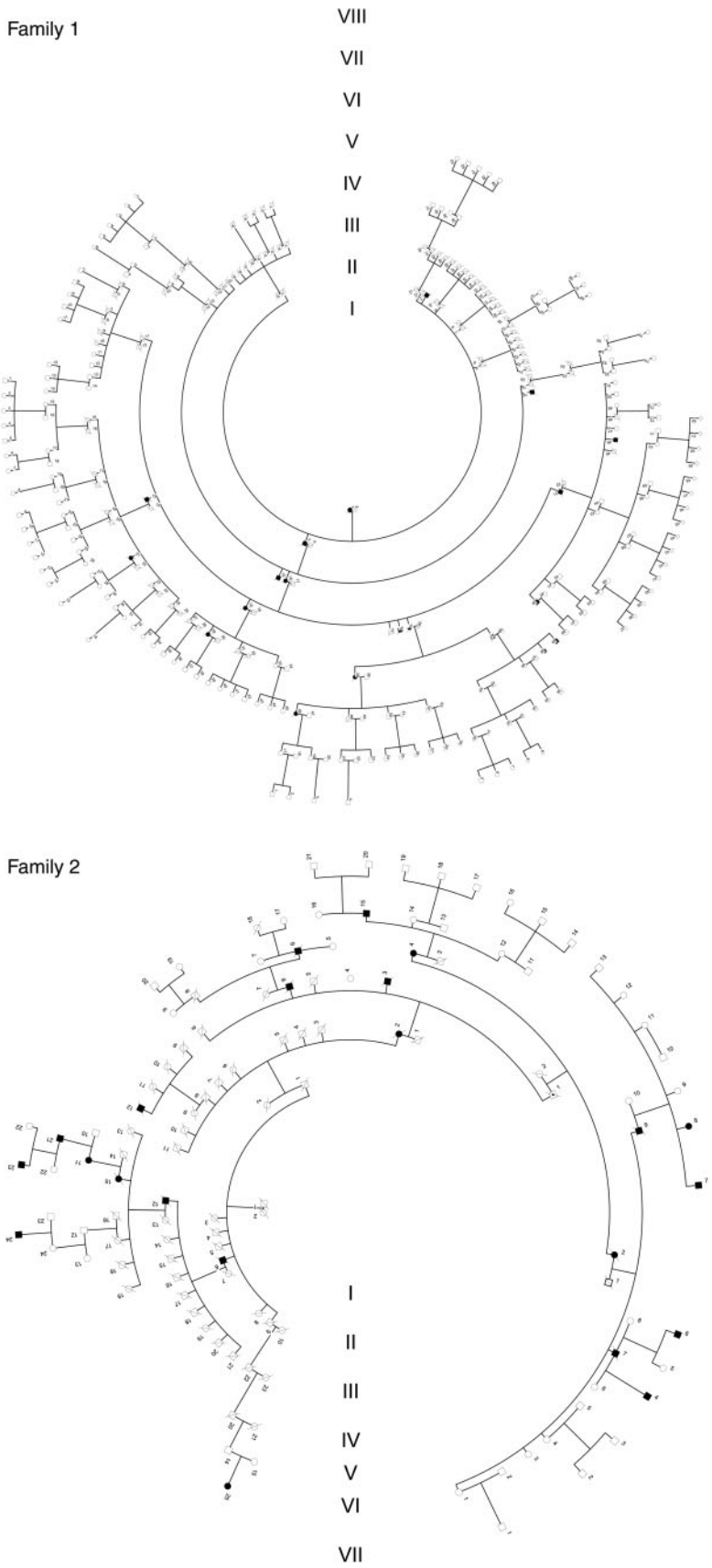

Fig. 4 Pedigrees of families. Families 1–5, index patients are described in Table 1.

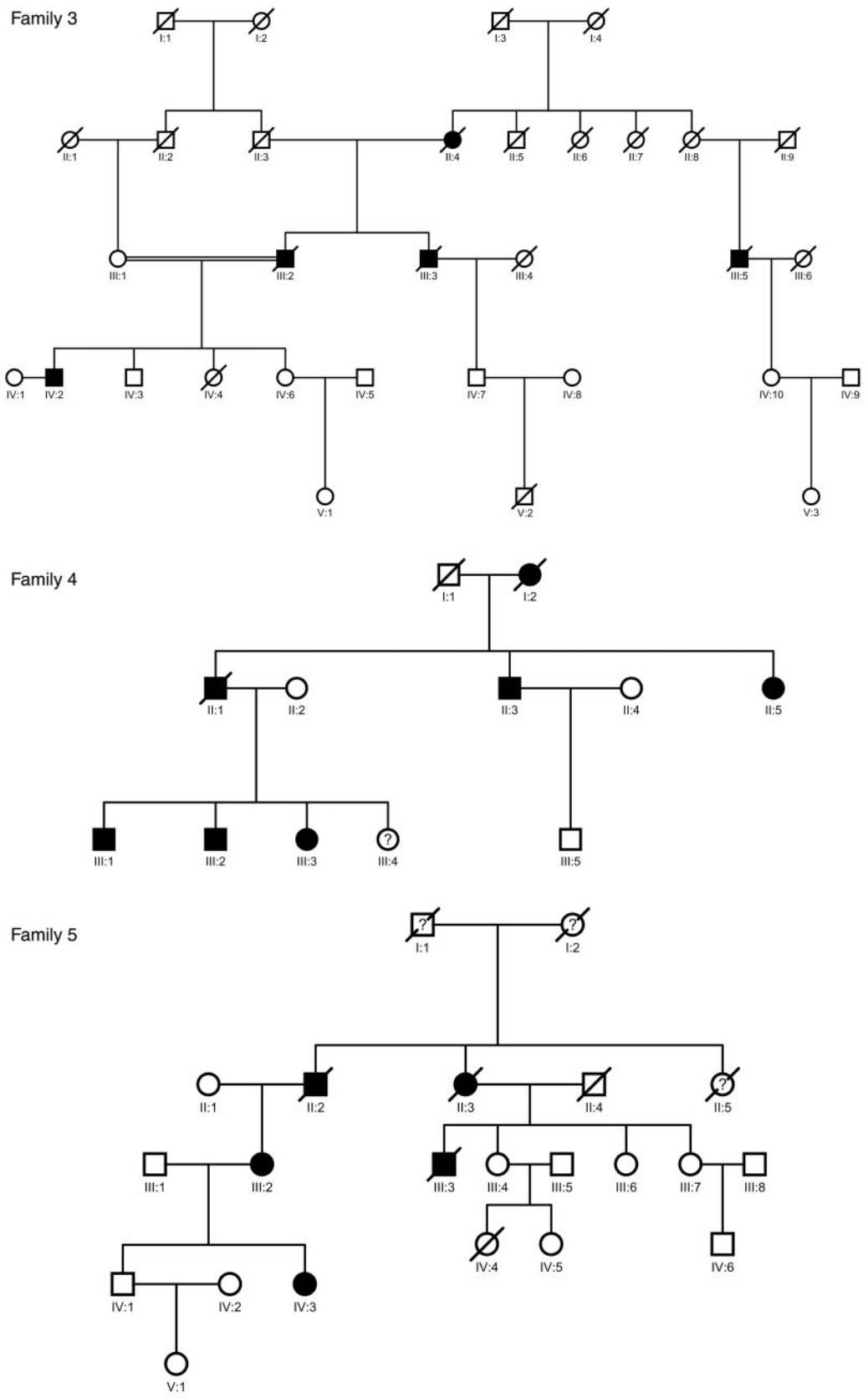

Fig. 4 Continued.

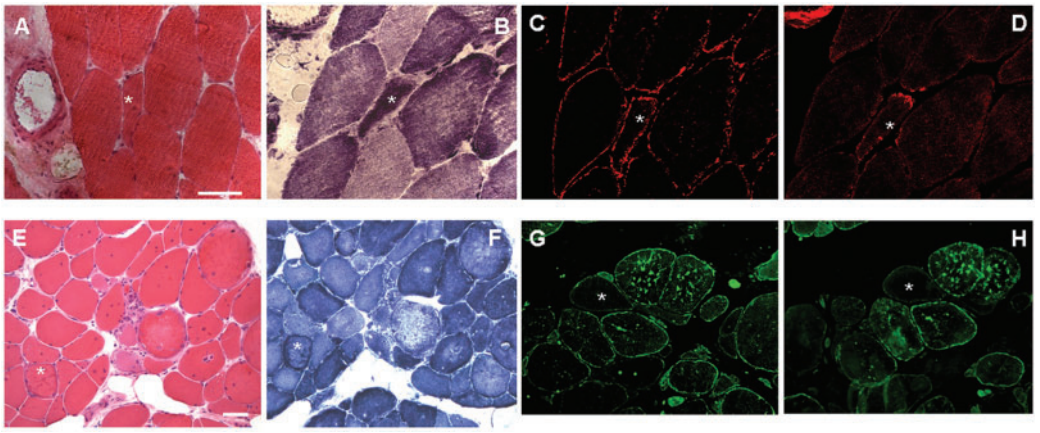

Fig. 5 Histological analysis and immunohistochemistry of muscle specimen of patient I (**A**–**D**) and patient I3 (**E** and **F**). Serial sections: (**A**) H&E: in addition to normal myofibres, rarely angulated atrophic fibres are found (asterisks). (**B**) NADH: dark angulated myofibres are suspicious for protein aggregates (asterisks). (**C**) Desmin staining reveals only limited positive staining of subsarcolemmal aggregates. (**D**) Filamin-c staining shows more pronounced marking of subsarcolemmal aggregates. (**E**) H&E: myopathic findings are shown, including increase in fibre-size variation, internalized myonuclei and rarely angulated atrophic fibres. (**F**) NADH: dark myofibres are suspicious for protein aggregates. Serial sections: (**G**) anti-desmin staining and (**H**) anti-alpha-B-crystallin staining show positive marking of subsarcolemmal and intracytoplasmic aggregates (asterisks indicate corresponding fibres). Bars in (**A**) and (**E**): 30 μm.

pattern with rounding of muscle fibres, increased fibre-size variability with diameters ranging from 16 to 153 μm (normal 40–80 μm), some necrotic and regenerating fibres, internalization of nuclei in 40% of the fibres and many fibres with basophilic inclusions which showed intense labelling with antibodies against desmin and αB-crystallin (Fig. 5).

Family 5—limb girdle phenotype

The 33-year-old female index patient (patient 14, Table 2, Fig. 4) noticed myalgia after exercise since age 16 years. At age 30 years, she noted first problems in climbing stairs. Around the same time she noticed an inability to walk on heels. Neurological examination at age 33 years revealed paresis of proximal leg muscles (MRC 4/5) and foot extensors (3/5), but full strength of foot flexors, arm and hand muscles. Lung function tests showed beginning respiratory involvement, cardiac function was normal. Whole body MRI scan showed marked atrophy and fatty degeneration of proximal and distal leg muscles, predominantly in the dorsal leg compartment, while in the upper extremities only deltoid muscles are mildly affected (Fig. 3).

The 64-year-old mother (patient 15, Table 2) of the index patient noted first symptoms at age 40 years in form of proximal leg weakness, foot drop and cardiac arrhythmia. At age 45 years additional weakness in shoulder girdle and arm muscles occurred, at age 55 years, finger flexors were also affected. From age 58 years, she used a walking frame, and is now wheelchair-bound. Meanwhile, her lung function deteriorated requiring non-invasive ventilation. A muscle biopsy was obtained 25 years ago and showed a vacuolar myopathy, suggestive of inclusion body myopathy. Ultrastructural and immunohistochemical analysis was not carried out.

Her father and a male paternal cousin both died from sudden cardiac death at age 53 and 56 years, respectively, the cousin was further reported to have suffered from muscle weakness.

Discussion

We report clinical, electrophysiological, histopathological and molecular data of 15 patients from five independent families affected with a dominant myopathy due to *DES* (R350P), presenting with a highly variable clinical and morphological phenotype. Up to now, more than 30 mutations in *DES* have been reported and some tentative genotype–phenotype correlations have been proposed based on the mutation site, inheritance pattern and clinical manifestations (Goldfarb *et al.*, 2004; Paulin and Li, 2004). However, no distinct clinical phenotype was found to be closely associated with a certain mutation, since most mutations were limited to a single or few families. Clinical manifestations in the autosomal dominant syndrome included distal or proximal progressive skeletal myopathy, cardiomyopathy and respiratory dysfunction alone or their combination (Goldfarb *et al.*, 2004). For the first time we describe a larger cohort of patients harbouring the same *DES* mutation (R350P). Large variability was recognized, (Table 3) even within the same family, ranging from scapuloperoneal ($n = 2$, 12%), limb girdle ($n = 10$, 60%) and distal phenotypes ($n = 3$, 18%) with variable cardiac ($n = 7$, 41%) or respiratory involvement ($n = 7$, 41%). In advanced stages of the disease, all muscles are affected, but the biceps bracchii is relatively spared in patients

Table 3 Clinical phenotype of DES R350P patients

	All patients ($n = 15$)	Males ($n = 9$)	Females ($n = 6$)
Mean age of onset (years)	40 ± 10	37 ± 5	46 ± 13
Onset proximal	11	6	5
Onset distal	4	3	1
Distal > proximal weakness	4	2	2
Proximal > distal weakness	8	4	4
Scapuloperoneal weakness	2	1	1
Swallowing problems	4	3	1
Facial weakness	2	1	1
Gynaecomastia		3	
Respiratory involvement	7	3	4
Cardiac involvement	7	3	4
Death < 60 years	3	2	1
CK level (normal < 160)	557 ± 145	739 ± 213	256 ± 131

with scapuloperoneal distribution. In addition, facial weakness, dysphagia and gynaecomastia were frequently observed.

Desminopathy (R350P) is inherited with an autosomal dominant pattern and shows full penetrance. Mean onset of disease of male patients was at age 37 years. In female patients, disease onset occurred later (mean at age 46 years), and progression seemed less severe. While males do not have a higher overall incidence of cardiac or pulmonary involvement compared to females, risk for sudden death or fatal respiratory failure seems to be higher in males: 2 males versus 1 female in the patients investigated for this study, and 12 males versus 2 females from families' historical data. Therefore, gender-related factors or modifier genes may be involved in determining disease onset and severity. Recently, similar gender-related phenotypic differences have been described in a Spanish desminopathy family due to a novel L370P mutation (Arias *et al.*, 2006).

Furthermore, it would be important to identify genetic associations responsible for heart and respiratory problems, like mutations in other 'neuromuscular' genes modifying the clinical manifestation or synergistically contribute to disease severity of desminopathy, which was recently shown for dominant Emery–Dreifuss muscular dystrophy by mutations in both emerin and desmin proteins (Muntoni *et al.*, 2006).

DES R350P is relatively frequent in German patients with dominant, adult-onset myopathies. Other populations were not represented in our cohort of patients. Since the mutation was detected in five unrelated families, we wondered whether position 350 is a mutation hotspot. In this case, the mutation would have arisen independently on divergent alleles. In contrast, we found the allele harbouring R350P in all five families associated with the same array of extragenic, polymorphic markers which spans at least 3 Mb. This argues strongly for a founder allele, i.e. the mutation arose only once several centuries ago to allow for the observed recombination events at adjacent flanking markers.

Usually, desminopathies are diagnosed through muscle histology. Pathological features in desminopathies consist of a myopathic pattern with variability of fibre-size, the presence of angulated atrophic fibres, internalized nuclei, rimmed and autophagic vacuoles, rarely cytoplasmic eosinophilic inclusions and accumulation of desmin in immunohistochemistry. Furthermore, in autophagic vacuoles myelin-like lamellae, aggregates, and IBM-like paired helical tubulofilaments were described (Vrabie *et al.*, 2005). Selcen *et al.* (2004) reported in a cohort of patients with myofibrillar myopathy an abnormal fibre-size variation, with some fibre diameters as small as 5 µm or as large as 150 µm and type-grouping in 70% of the muscle specimens. In most cases, however, atrophic fibres accounted for only a small proportion of the total. Furthermore, neurogenic-like changes are described in other myofibrillar myopathies such as myotilinopathy (Selcen and Engel, 2004) and ZASPopathy (Selcen and Engel, 2005).

In 1965, Kaeser reported a kindred with atrophies and weakness in a scapuloperoneal distribution following an autosomal dominant trait. Histopathological examination at autopsy in one patient, the father of our index patient (patient 1, family 1), revealed fibre-size variation ranging from 12 to 54 µm, increase in muscle nuclei and small muscle fibres with varying shapes (some very small and round, others polyhedral) found in biceps, quadriceps, interosseus dorsalis and thenar muscles (Probst *et al.*, 1977). However, clear myofibrillar changes are not reported. Ultrastructural findings were described as 'a juxtanuclear inclusion which consisted of a crystal-like lattice of dense particles', that may resemble protein aggregates. Retrospectively, there is considerable overlap with the myopathological changes described in myofibrillar myopathies. Kaeser suggested a neurogenic origin, but demonstrated significant differences from the cases reported by Davidenkow (1939). Neuropathic scapuloperoneal syndrome (Davidenkow's syndrome) was recently found associated to chromosome 17p11.2 deletions (Verma, 2005). Interestingly, the current histopathological examination of the biceps brachii muscle of patient 1 showed very limited myofibrillar changes or protein aggregation which were only picked up by desmin and filamin c immunohistochemistry. Therefore, we suggest to proceed with genetic analysis in adult-onset, dominant myopathies even in the absence of typical, myofibrillar changes upon histological examination, once other causes of dominant myopathies have been excluded.

Our findings allow re-assigning the scapuloperoneal syndrome in the kindred originally described by Kaeser to the group of dominant myopathies, due to the R350P desmin mutation. Future studies will have to elucidate how other factors and modifier genes may influence the severity, penetrance and expression of the disease. Identification of these genes and pathways may lead to novel therapeutic strategies.

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References

Arias M, Pardo J, Blanco-Arias P, Sobrido MJ, Arias S, Dapena D, et al. Distinct phenotypic features and gender-specific disease manifestations in a Spanish family with desmin L370P mutation. Neuromuscul Disord 2006; 16: 498–503.

Ariza A, Coll J, Fernandez-Figueras MT, Lopez MD, Mate JL, Garcia O, et al. Desmin myopathy: a multisystem disorder involving skeletal, cardiac, and smooth muscle. Hum Pathol 1995; 26: 1032–7.

Bar H, Fischer D, Goudeau B, Kley RA, Clemen CS, Vicart P, et al. Pathogenic effects of a novel heterozygous R350P desmin mutation on the assembly of desmin intermediate filaments in vivo and in vitro. Hum Mol Genet 2005; 14: 1251–60.

Davidenkow S. Scapuloperoneal amyotrophy. Arch Neurol Psychiat 1939; 41: 694–701.

Goebel HH. Desmin-related neuromuscular disorders. Muscle Nerve 1995; 18: 1306–20.

Goldfarb LG, Park KY, Cervenakova L, Gorokhova S, Lee HS, Vasconcelos O, et al. Missense mutations in desmin associated with familial cardiac and skeletal myopathy. Nat Genet 1998; 19: 402–3.

Goldfarb LG, Vicart P, Goebel HH, Dalakas MC. Desmin myopathy. Brain 2004; 127: 723–34.

Goudeau B, Dagvadorj A, Rodrigues-Lima F, Nedellec P, Casteras-Simon M, Perret E, et al. Structural and functional analysis of a new desmin variant causing desmin-related myopathy. Hum Mutat 2001; 18: 388–96.

Horowitz SH, Schmalbruch H. Autosomal dominant distal myopathy with desmin storage: a clinicopathologic and electrophysiologic study of a large kinship. Muscle Nerve 1994; 17: 151–60.

Kaeser HE. Scapuloperoneal muscular atrophy. Brain 1965; 88: 407–18.

Kaminska A, Strelkov SV, Goudeau B, Olive M, Dagvadorj A, Fidzianska A, et al. Small deletions disturb desmin architecture leading to breakdown of muscle cells and development of skeletal or cardioskeletal myopathy. Hum Genet 2004; 114: 306–13.

Li D, Tapscoft T, Gonzalez O, Burch PE, Quinones MA, Zoghbi WA, et al. Desmin mutation responsible for idiopathic dilated cardiomyopathy. Circulation 1999; 100: 461–4.

Muntoni F, Bonne G, Goldfarb LG, Mercuri E, Piercy RJ, Burke M, et al. Disease severity in dominant Emery Dreifuss is increased by mutations in both emerin and desmin proteins. Brain 2006; 129: 1260–8.

Park KY, Dalakas MC, Goebel HH, Ferrans VJ, Semino-Mora C, Litvak S, et al. Desmin splice variants causing cardiac and skeletal myopathy. J Med Genet 2000a; 37: 851–7.

Park KY, Dalakas MC, Semino-Mora C, Lee HS, Litvak S, Takeda K, et al. Sporadic cardiac and skeletal myopathy caused by a de novo desmin mutation. Clin Genet 2000b; 57: 423–9.

Paulin D, Li Z. Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. Exp Cell Res 2004; 301: 1–7.

Probst A, Ulrich J, Kaeser HE, Heitz P. Scapulo-peroneal muscular atrophy. Full autopsy report. Unusual findings in the anterior horn of the spinal cord. Lipid storage in muscle. Eur Neurol 1977; 16: 181–96.

Schroder R, Goudeau B, Simon MC, Fischer D, Eggermann T, Clemen CS, et al. On noxious desmin: functional effects of a novel heterozygous desmin insertion mutation on the extrasarcomeric desmin cytoskeleton and mitochondria. Hum Mol Genet 2003; 12: 657–69.

Selcen D, Engel AG. Mutations in myotilin cause myofibrillar myopathy. Neurology 2004; 62: 1363–71.

Selcen D, Engel AG. Mutations in ZASP define a novel form of muscular dystrophy in humans. Ann Neurol 2005; 57: 269–76.

Selcen D, Ohno K, Engel AG. Myofibrillar myopathy: clinical, morphological and genetic studies in 63 patients. Brain 2004; 127: 439–51.

Sjoberg G, Saavedra-Matiz CA, Rosen DR, Wijsman EM, Borg K, Horowitz SH, et al. A missense mutation in the desmin rod domain is associated with autosomal dominant distal myopathy, and exerts a dominant negative effect on filament formation. Hum Mol Genet 1999; 8: 2191–8.

Sugawara M, Kato K, Komatsu M, Wada C, Kawamura K, Shindo PS, et al. A novel de novo mutation in the desmin gene causes desmin myopathy with toxic aggregates. Neurology 2000; 55: 986–90.

van der Ven PF, Obermann WM, Lemke B, Gautel M, Weber K, Furst DO. Characterization of muscle filamin isoforms suggests a possible role of gamma-filamin/ABP-L in sarcomeric Z-disc formation. Cell Motil Cytoskeleton 2000; 45: 149–62.

Verma A. Neuropathic scapuloperoneal syndrome (Davidenkow's syndrome) with chromosome 17p11.2 deletion. Muscle Nerve 2005; 32: 668–71.

von der Hagen M, Schallner J, Kaindl AM, Koehler K, Mitzscherling P, Abicht A, et al. Facing the genetic heterogeneity in neuromuscular disorders: linkage analysis as an economic diagnostic approach towards the molecular diagnosis. Neuromuscul Disord 2006; 16: 4–13.

Vrabie A, Goldfarb LG, Shatunov A, Nagele A, Fritz P, Kaczmarek I, et al. The enlarging spectrum of desminopathies: new morphological findings, eastward geographic spread, novel exon 3 desmin mutation. Acta Neuropathol (Berl) 2005; 109: 411–7.